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ON THE CAUDAL NEUROSECRETORY SYSTEM OF THE TELEOST FISH, *FUNDULUS HETEROCLITUS* L.

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INTRODUCTION

It was early observed that a bulb-like structure was associated with the terminal portion of the spinal cord of certain fishes (Weber 1827, Rauber 1877, Arsaky 1813, Ziehen 1903, and Favaro 1925). This outgrowth of the spinal cord of different systematical groups of teleosts was described by Favaro (1925), who studied a large number of species. Because of the histological and morphological resemblance of this organ to the neurohypophysis, he named it "Ipofisi caudale."

Secretory cells in the terminal portion of the spinal cord were described first by Dahlgren (1914), and later by Speidel (1919, 1922). The latter also gave a histological description of some "irregular glandular cells," sometimes called "Dahlgren's cells," in the terminal portion of the spinal cord of certain elasmobranchs and teleosts. Recently Enami (1955 a) investigated the caudal portion of the spinal cord of the eel, *Anguilla japonica*, with respect to the secretory activity of "Dahlgren's cells" and found that neurosecretory cells were present in the terminal portion of the spinal cord. They extended caudally from the level of the last sixth or seventh vertebra. These neurosecretory cells, according to Enami, resembled the secretory cells of the hypothalamus, and had axons which served as neurosecre-

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tory pathways. The neurosecretory material was released at nerve endings, which terminate in a ventral outgrowth of the spinal cord. The outgrowth served as a storage depot for secretory material. This structure, which was named "Ipofisi caudale" by Favaro (1925), was called "Neurohypophysis spinalis" or "Urohypophysis" by Enami (1955 a).

These observations were extended by Enami and Imai (1955, 1956 a and b), with descriptions of the caudal neurosecretory system of a number of freshwater and marine teleosts. Recently Sano (1958 a and b) described the "Neurophysis" and the neurosecretory system of the spinal cord of *Tinca vulgaris* and a number of other teleosts. It has been suggested that the probable function of the caudal neurosecretory system would be osmoregulation (Enami, Miyashita and Imai 1956, and Holmgren 1958 b).

Terminology. Different names have been suggested for the above-mentioned outgrowth of the spinal cord, which serves as a storage organ for the neurosecretory material. Because the organ may be located either dorsal, ventral, or lateral to the spinal cord (Favaro 1925, Sano 1958 b), and since the term "neurohypophysis" has already been used for the "pars nervosa" of the pituitary gland, the name "neurohypophysis spinalis" is not adequate. In order to stress the fact that the organ is an outgrowth of the terminal portion of the spinal cord the name *urophysis spinalis*¹ has been adopted for this study. This name stresses the position of the organ relative to the body, regardless of its varying position in relation to the spinal cord.

Material and methods. Twenty specimens of the teleost fish, *Fundulus heteroclitus* L., standard length 4-6 cm., caught during different times of the year, were used for the anatomical description. Twelve embryos were also used in an attempt to determine the mode of development of the urophysis. For the anatomical and histological descriptions, the terminal portion of the spinal cord, including the adjoining vertebral column, was fixed in Bouin's solution to which 1 per cent of CaCl₂ had been added; Zenker's, Carnoy's and Orth's fixing fluids were also used. The tails were decalcified in 7 per cent HNO₃ and afterwards treated in 10 per cent Na₂SO₄, and the sections were cut at 4-8 microns, sagittally and transversely, using the usual paraffin technique.

¹ Suggested by Dr. G. Fridberg, Zoological Institute, Stockholm.

The following staining techniques were used: Gomori's haematoxylin-phloxin method, Heidenhain's azan, Halmi's aldehyde fuchsin following performic acid oxidation (Holmgren 1958 a), Mallory's connective tissue stain, Bodian's protargol method, Masson's trichrome stain according to Gomori (1950), periodic acid-Schiff (PAS), and alcian blue.

DESCRIPTION

Location of the urophysis spinalis. The urophysis in *Fundulus* is observed as a round body, easily visible under the dissecting microscope. It lies at the end of the spinal cord, ventral to the terminal portion, above the articulation between the last vertebra and the hypural bone. This location of the urophysis (Figs. 1-4) is similar to that described for a great number of teleosts by Enami and Imai (1956 a and b), and Sano (1958 a and b). These authors also found that the urophysis is generally situated ventral to the spinal cord.

Morphology and histology. According to descriptions by Favaro (1925), the urophysis consists mainly of modified glial tissue. The blood vessels present in the organ enter from the connective tissue mantle which surrounds the spinal outgrowth. Rauber (1877), on the other hand, believed that the urophysis consisted mainly of connective tissue. Enami (1955 a) found both connective tissue and modified glial tissue in the organ, and stressed the similarity of the urophysis to the neurohypophysis of vertebrates and the sinus gland of Crustacea.

The urophysis spinalis of *Fundulus heteroclitus* is a very conspicuous structure measuring about 0.2×0.3 mm., which approximately corresponds to the size of the pituitary gland. It is covered by a heavily pigmented leptomeninx. The histological elements distinguished in the urophysis are mainly of three kinds. In the dorsal region, *nerve fibers* are found which emanate from the neurosecretory cells, situated in the terminal portion of the spinal cord (Fig. 1). Orange G positive Herring body-like secretory droplets are present at the end of these axons (Fig. 3). These 'secretory droplets' are probably dilated nerve endings. The colloidal masses within the dilated nerve endings stain red in Gomori's chrome haematoxylin-phloxin, thus showing affinity

for the phloxin component. The staining reaction with azan is bluish-red. The nerve tracts are usually not distributed all over the organ as observed in some other species but merely confined to the dorsal region of the urophysis.

The urophysis is very vascular with blood vessels distributed throughout the organ (Figs. 4, 6). These vessels are of sinusoid type with thin walls, supported by argyrophilic connective tissue as shown with Bodian's protargol method. In the dorsal region of the urophysis the blood vessels frequently seem to surround

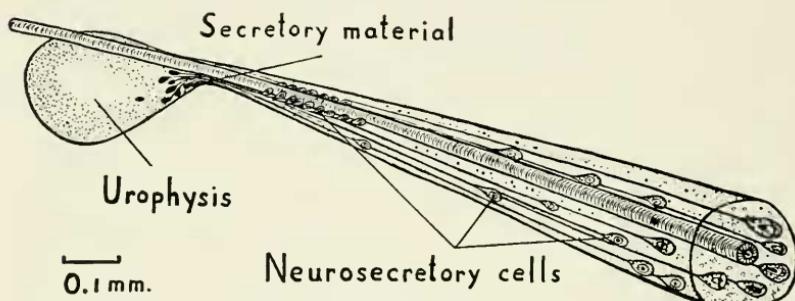


Fig. 1. Schematic picture of the caudal neurosecretory system of the teleost, *Fundulus heteroclitus* L.

intercellular spaces, which are most conspicuous in the area where the secretory tracts terminate. Besides the nerve endings, the urophysis consists of *argyrophilic connective tissue* and *neuroglia*. The glial cells contain very little cytoplasm, sometimes consisting only of small nuclei and surrounding membranes. The differentiation between the different types of tissue elements is especially well observed after using Heidenhain's stain. The distribution of the blood vessels indicates that the secretory material is taken up by the blood.

Transverse sections show that the urophysis is surrounded by a sella turcica-like connective tissue capsule (Fig. 4). This extension of the leptomeninx is sometimes very heavily invested with melanophores. In other teleosts, the urophysis may be attached to the spinal cord by means of a stalk, which sometimes is very conspicuous (Enami and Imai 1956 a and b, Sano 1958 b).

The urophysis of *Fundulus heteroclitus* is very closely attached to the spinal cord by means of a stalk with a broad base. Most of the organ, however, is separated from the spinal cord. Cross-sections of the urophysis (Fig. 4) show a well defined space between the dorsal part of the organ and the ependymal parts of the spinal cord. Reissner's fiber is still present in this thin terminal portion of the spinal cord.

In the region where the spinal cord axons pass into the urophysis the secretory droplets are especially frequent (Fig. 3). The secretory material within the dilated nerve endings is here homogeneous and appears colloidal. The staining reaction also seems to have changed in a 'basophilic' direction (cf. below). Some of the secretory droplets were slightly aldehyde fuchsin-positive in the dorsal region of the urophysis, but the neurosecretory cells stained negatively.

The neurosecretory cells. The neurosecretory cells are present in the terminal portion of the spinal cord, appearing first at the level of the sixth vertebra from the caudal end and most concentrated above the third vertebra and caudally (Figs. 1, 5). The secretory cells, which contain Nissl substance, are obviously specialized nerve cells of multipolar, bipolar, and also unipolar type. The neurosecretory cells stain negatively with both Gomori's chrome haematoxylin and Halmi's aldehyde fuchsin. Instead, they take up the phloxin component of Gomori's stain and also show great affinity for the acid fuchsin of Mallory's stain, thus confirming the findings of Enami (1955 a).

The neurosecretory cells are also stained with the azocarmine of Heidenhain's stain thus showing a marked 'acidophilic' reaction which may be due to secretory material in the cytoplasm (cf. below). The spinal neurosecretory cells were observed to contain scattered small PAS-positive inclusions, but the secretory droplets within the urophysis stained negatively. A positive reaction would have denoted the presence in the secretion of considerable amounts of polysaccharides, mucopolysaccharides, glucoproteins or glycolipoids (Lillie 1954). The negative reaction to PAS agrees with the findings of Sano (1958b).

The neurosecretory cells have various sizes and shapes. Those which are situated closer to the urophysis, are generally smaller. The secretory cells are generally distributed all over the spinal

cord which in the terminal portion does not show the usual separation into white and gray material. The secretory cells were found to contain a large nucleus with an irregular shape, as already indicated in the early descriptions by Speidel (1919, 1922). The size of the neurosecretory cells in *Fundulus*, however, was considerably less than the originally described spinal cells in skates and the eel (Speidel 1919, Enami 1955a). The secretory material usually contained very fine granules, and was generally distributed all through the cytoplasm but sometimes was merely confined to the periphery of the cells. The irregular nucleus contains one or more nucleoli and fine granular chromatin.

In the sections stained with Heidenhain's azan, it was easy to distinguish the neurosecretory cells, with their 'acidophilic' reaction, from the strongly 'basophilic' motor cells. Because of the irregular shape of the nuclei, sometimes two or several nuclear areas were visible when a cell was cut in a certain plane. In the cytoplasm of the secretory cells the 'acidophilic' material was observed in various amounts, indicating a cycle in the formation of the secretion. The 'acidophilic' reaction was interpreted as a measure of the secretory activity. Nerve cells in general and some neurosecretory ones from the hypothalamic system may show a 'basophilic' reaction (Scharrer and Scharrer 1954), perhaps due to a continuous synthesis of proteins, hormones and high amounts of ribonucleic acid. Observations on cells in different stages of secretion have indicated that the cell in the beginning of the secretory phase does not show a considerable 'acidophilic' reaction. As secretion begins the nuclei of the cells begin to enlarge and 'acidophilic' granulated material arises in the area around the nucleus. Later, the secretory material is often found in the periphery of the cells or distributed all through the cytoplasm. It has been suggested that the heavy 'acidophilic' reaction particularly in the periphery of the neurosecretory cells is due to accumulation in these areas of 'acidophilic' material and Nissl-substance (Sano 1958 b). The observations in this study support the suggestion by Sano (1958 b) that the nucleus takes an active part in the formation of the secretory material. No selective stain is so far known for the secretory material.

The secretory material of the nerve cells is transported in the axons which serve as neurosecretory pathways and is stored in dilated nerve endings and released in the dorsal region of the urophysis where the axons terminate. Observations were made on the staining reaction of the secretory droplets to acid alcian blue stain. The observed negative reaction would mean that the secretion does not contain disulfide groups. These observations agree with those by Sano (1958 b), who found that the secretion in the caudal neurosecretory system of *Tinca vulgaris* reacted negatively for the astra blue and alcian blue stains.

In the limited material examined (20 species), obtained during fall and winter, no pronounced seasonal differences were observed in secretory activity.

Development of the urophysis spinalis. The caudal neurosecretory cells and the urophysis spinalis were not developed in the stages immediately after hatching, length 15-20 mm. (Fig. 7). The hypothalamus-pituitary system was apparently actively secreting at this stage. These observations thus confirm the findings by Favaro (1925) and Sano (1958 b) that the urophysis spinalis and the caudal neurosecretory system develop late during the ontogeny. Sano (1958 b) noted that the urophysis spinalis was still not developed in *Salmo fario* at a size of 2.5-3 em. In *Anguilla* of 6 em. length, the caudal neurosecretory system had begun to function.

DISCUSSION

Many investigators have pointed out the similarity in structure between the posterior pituitary and the urophysis spinalis, and this analogy has also been stressed in the names suggested, e.g. "Ipofisi caudale" (Favaro 1925), "Neurohypophysis spinalis" (Enami 1956), and "Neurophysis spinalis caudalis" (Sano 1958 a, and Sano and Hartmann 1958). This similarity in structure and organization of the urophyseal system compared to the hypothalamic system was emphasized in this study by the fact that the secretory material in the urophysis was stored in dilated nerve endings.

As already mentioned above, there is no selective stain for the secretory material in the caudal neurosecretory system, although the dilated nerve endings show affinity for the orange G com-

ponent of various stains. There is, however, reason to believe that the 'acidophilic' reaction of the cells is due to the presence of secretion. The terms 'acidophilic' and 'basophilic' as used in this study are relative, depending on fixation, staining and other treatment. The two terms have been used to indicate that the number of either acid carboxyl or basic amino groups of the protein molecule may be predominant (Romeis 1948). These terms may be useful in this case for descriptive purposes until a more selective stain for the eandal neurosecretory system has been developed.

There are reasons to believe that the neurosecretory cells in the teleost spinal cord, although they differ in size from the cells originally described by Dahlgren (1914) and Speidel (1919), are identical and homologous with those in the skates, in spite of the fact that Speidel (1922) did not find the corresponding cells in the spinal cord of *Fundulus*. The neurosecretory cells of *Fundulus* do not differ greatly from unmodified nerve cells, except for their staining reactions (cf. above). They are far less conspicuous and appear less "glandular" than the secretory cells of the skates and the eel and can therefore be readily overlooked.

Sano (1958 b) found that the neurosecretory material inside the axons could not be a pathological product of the cells or of the myelin sheath but must be considered as a true neurosecretion, as earlier indicated by Enami (1955 a, 1956). This study can further support these opinions by showing the conspicuous, Herring body-like, secretory droplets which are formed at the nerve endings in the dorsal region of the urophysis spinalis (Fig. 3). These secretory droplets are, according to this study, the dilated nerve endings, which serve to store the secretory material. The secretory material is thus generally not stored in the urophyseal tissues (cf. Enami 1955 a).

The origin of the secretory granules inside the neurosecretory cells has been extensively dealt with by Scharrer (1934), Palay (1943), Hild (1950), Scharrer and Scharrer (1954), Enami (1955 b), and others. According to these descriptions, the formation of the secretion is not fully understood. It has been observed that granules also are present in the nucleus of certain cells of the nucleus tuberalis lateralis of teleosts (Scharrer 1934, Stahl and Seite 1955, Enami 1955 b, Ortmann 1956, Stahl 1957).

According to Stahl and Seite (1955), the intranuclear granules probably cannot be connected with neurosecretion. Enami (1955 b), on the other hand, considered that the 'acidophilic' granules in the nucleus entered the cytoplasm through the nuclear membrane into the area around the nucleus or were emitted into neurites. The direct role of the nucleus in the secretory process has not yet been clearly demonstrated. As far as the caudal neurosecretory system is concerned, Sano (1958 b) believes that the nucleus very likely takes an important part in the formation of the secretion. The opinion of Scharrer and Scharrer (1954) and Enami (1955) that the secretory material may be formed out of the Nissl substance or at the expense of the latter, could not be confirmed in this study. As mentioned above, it was observed that the secretory material seemed to originate from the area around the nucleus, which was interpreted to mean that the nucleus plays an important role in the formation of the secretion.

It was previously noted that the secretion in the neurosecretory cells reacted negatively with Gomori's chrome haematoxylin and Halmi's aldehyde fuchsin. The secretory droplets (colloid masses) in the dorsal region of the urophysis (Fig. 3), on the other hand, showed some affinity for the aldehyde fuchsin. This difference in staining reaction with aldehyde fuchsin in the secretory cells as compared to the urophyseal colloid may indicate that physical or chemical changes have occurred in the secretory material in association with its storage in the nerve endings. A stronger reaction to Gomori's stain is also generally observed in the hypothalamus-pituitary system for the Herring bodies compared with the neurosecretory cells or the tracts. In the hypothalamus-pituitary system, the presence of a stainable carrier substance or prosthetic group, has been postulated (Schiebler 1951). If biochemical changes do occur at the release of the neurosecretory material at the nerve endings, it would affect the response to certain dyes.

The negative reaction of the caudal neurosecretory cells and the secretory material in the urophysis to the periodic-acid-Schiff stain is in contrast with the positive reaction in the hypothalamus-pituitary system. Schiebler (1951) found that the neurosecretory cells of the hypothalamus of the teleost *Esox* were strongly periodic-Schiff positive.

The studies by Enami (1955 a) and Sano (1958 a and b) have demonstrated the presence in fishes of a caudal neurosecretory system which morphologically is organized in much the same manner as the hypothalamus-pituitary system in vertebrates and the intercerebralis-corpus cardiacum system in insects, all showing characteristic synthesizing, transporting, and release-storage elements. Such a neurosecretory system is, according to this study, present and actively secreting in the teleost fish, *Fundulus heteroclitus* L.

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SUMMARY

An examination of the terminal portion of the spinal cord of the teleost *Fundulus heteroclitus* L. has confirmed the presence of a caudal neurosecretory system. The neurosecretory cells are distributed from and posterior to the sixth vertebra and are more concentrated above the third vertebra from the caudal end of the spine. Histologically, these neurosecretory cells are modified nerve cells of multi-, bi-, and unipolar type. They show affinity to 'acidophilic' stains such as acid fuchsin, phloxin, and azoearmine. In contrast with the neurosecretory cells of the hypothalamus, these spinal cells are Gomori-negative and show no reaction for the alcian blue stain, the latter staining reaction indicating the absence in the secretion of sulfonate groups. The secretory material, which also shows negative periodic-acid-Schiff reaction, is transported from the neurosecretory cells in axons serving as secretory pathways, and the neurosecretion is stored in dilated nerve endings which terminate in a conspicuous organ, the *urophysis spinalis* (neurohypophysis spinalis caudalis, neurophysis, urohypophysis), which is a ventral outgrowth of the spinal cord. At the nerve endings, Herring body-like secretory droplets are formed. No seasonal changes in the secretory activity of the caudal neurosecretory system were observed. The urophysis shows histologically the same structure as the neurohypophysis

of the pituitary gland, containing nerve endings, modified glial tissue and blood vessels.

The urophysis and the caudal neurosecretory system must develop rather late during the ontogeny since it is not present in embryos immediately after hatching, at which time the hypothalamic-pituitary system is actively secreting.

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